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Chromosome Studies in the Myriapoda VI. A Study on the
Sex-chromosomes in Two Allied Species of Chilopods

With 19 Text-figures

Kazuo OGAWA

Hamamatsu Kita Koto-Gakko, Shizuoka-ken, Japan

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Since the pioneer work by Henking (1891), the efforts of animal cytologists have been devoted to chromosome research in relation to sex determination during a period of over five decades. A large part of the work has been carried out in insects because of their suitability for research work of this sort, and a large amount of valuable data has been accumulated. In most orders of insects, male heterogamety has been established on a large scale, with the exception of Lepidoptera and Trichoptera in which female heterogamety occurs (for a review, see Makino's list 1950). Lately Niiyama (1937, 1938, 1941, 1950) has published excellent papers which prove the occurrence of male heterogamety in Crustacea, a group of arthropods related to insects. Quite recently Hackman (1948), and Suzuki and Okada (1950) have demonstrated the same sex mechanism to occur in Arachnida, another group of arthropods. Nothing definite has been known, however, about the sex mechanism of chromosomes in Myriapoda, though there are some discrepant statements by earlier workers. Medes (1905), Bouin and Ancel (1911), and Bouin (1934) reported the occurrence of an X-0 mechanism in some species of *Scutigera* (Chilopoda), but the X element indicated by them is, according to Makino and Niiyama (1942), simply an autosome which escaped from the equatorial plate as the result of inadequate fixation. The present author has been engaged in a study of the chromosomes of chilopods in recent years, and has had an opportunity to observe some clear-cut evidence showing male heterogamety in two species, the data from which constitute the material of the present paper.

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Material and Method: The present study deals with the chromosomes of *Thereuonema hilgendorfi* and *Thereuopoda clunifera*. The testes, ovaries and digestive canals were fixed in Carother's fluid and modified Navashin's solution. The slides were prepared by the usual paraffin technique and stained after Heidenhain's iron-haematoxylin method.

I. The Chromosomes of *Thereuonema hilgendorfi* Verhoeff

This species is widely distributed over Japan-Hondo through Shikoku, Korea and Formosa. The material studied was obtained in the vicinity of Hamamatsu. The chromosomes of this species were reported preliminarily by the author (Ogawa 1950). In the present paper a more detailed account will be given with special reference to the sex chromosomes.

The diploid number of chromosomes was established to be 36 for both male and female cells (Figs. 1-4). Among the diploid elements, 34 are minute spheroidal ones with a slight variation of size, while the remaining two are very prominent in their elongated form of huge size in striking contrast to the former elements. The two always occupy a peripheral position in the equatorial plate, surrounded by the minute elements; they lie with their entire length manifesting some undulations. At a glance, the structural demarcation between the minute and elongated elements is very striking. Noticeably, these two exhibit a distinct size difference. The large one shows a segmentation into three parts, the smaller, into two (Figs. 1-2).

The female diploid complement consists also of 34 minute spheroidal and two elongated elements of undulating appearance (Figs. 3-4). The general configuration of the chromosomes is nearly like that of the male, although close study reveals that the two elongated chromosomes are identical in size in the female cell, whereas in the male they differ in length. Thus it follows that these two elongated chromosomes show a sexual difference. Accordingly, it is evident that the elongated elements are the sex chromosomes of this species, showing that the XY-mechanism occurs in the male and an XX-mechanism in the female.

Every metaphase plate of the primary spermatocytes shows 18 well-defined elements which are all bivalent in nature (Figs. 5-6). Of these 18 elements, 17 are small with slight variations in size; they are evidently derivatives from the 34 minute spheroidal elements of the



Figs. 1-11. Chromosomes of *Thereuonema hilgendorfi* Verhoeff. 1, Spermatogonial prometaphase. 2, Somatic metaphase in male digestive canal. 3-4, Somatic metaphases in female digestive canal. 5-6, Primary spermatocyte metaphases. 7, Primary spermatocyte metaphase, lateral view. 8, Primary spermatocyte anaphase. 9-10, Secondary spermatocyte metaphases. 11, Secondary spermatocyte anaphase. $\times 3800$.

diploid group. The remaining one is prominent among the others on account of its enormous size and unusual shape. Considered from its structural configuration it is undoubtedly the XY complex. It consists of a large X element and a smaller Y in an end-to-end conjugation (Figs. 5-7). It manifests a slight bending at the point where the two elements come in contact. It lies with its entire length in the equatorial plate, surrounded by the small ordinary bivalents. Sometimes a longitudinal splitting is observable along the entire length of the XY complex (Fig. 7).

Noticeable are the mode of segregation of the XY complex and its behaviour during the anaphase stage of the first division. As noted above, the X and Y elements come together in end-to-end association. At metaphase the XY complex manifests a pronounced longitudinal

split throughout its length along a plane parallel to the equatorial plate. From this fact it may be expected that the XY complex will be split into identical halves in the equatorial direction, and further examination confirms this expectation. At the beginning of the first division, the XY complex separates into two equal halves, identical in shape and size. This is clearly shown in Figures 7-8. With the further separation of the chromosomes, both halves of the XY complex, together with those of the autosomal elements, begin to migrate to the poles. Figure 8 shows the telophase configuration of the first division, in which the equational division of the XY complex is clearly indicated. Thus, in the first division, the XY bivalent gives rise to two identical XY dyads, corresponding in shape and size to each other. In the light of this finding it is beyond question that the XY complex divides equationally in the first meiotic division.

Another noticeable feature of the sex chromosomes is that in the anaphase each of the separated halves of the XY complex runs to the pole with its long axis parallel to the equatorial plate (Fig. 8). From this behaviour it may be concluded that the spindle fibres attach to the entire length of the XY complex. In other words, the XY complex carries diffused kinetochores.

Since the autosomal bivalents and the XY complex each divide into equal halves in the first division, it is to be expected that all the secondary spermatocytes will be provided with similar sets of chromosomes, the individual ones being identical in number and morphological characters. This expectation is substantiated by finding that every secondary spermatocyte shows 18 elements with the XY complex identical in all (Figs. 9-10). The XY complex is again very prominent among the autosomes on account of its characteristic configuration, composed of the larger X and the smaller Y in end-to-end conjugation. The X element has three segments and the Y, two. But the X and Y are single structures without longitudinal splitting, representing a dyad nature.

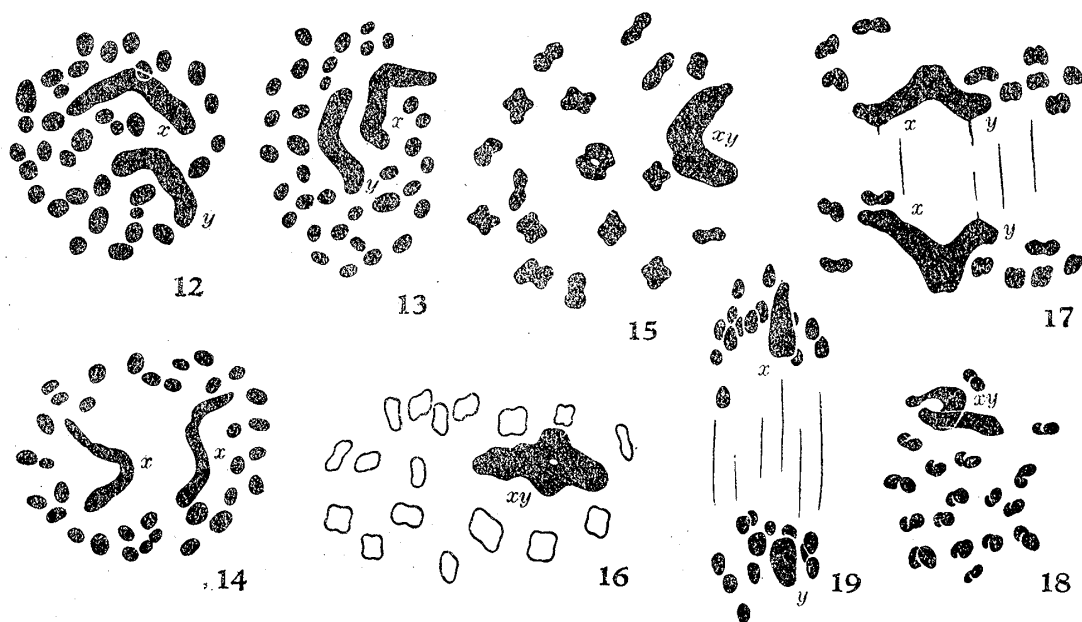
At anaphase of the second meiotic division, all of the autosomal elements separate each into two identical monads, while the XY complex always segregates into its component elements, the X and Y. A clear picture of this is depicted in Figure 11. In the light of this evidence, there is no doubt that it is in the second division that the X disjoins from the Y. The constant segregation of the X and Y in the second division results in the production of two kinds of spermatids, one having an X and the other a Y chromosome.

II. The Chromosomes of *Thereuopoda clunifera* (Wood)

According to Takakuwa (1943), the present species is widely distributed through Formosa, Kyushu, Shikoku, and Honshu, being abundant especially in the vicinity of Izu. The specimens used as material in this study were obtained at Bentenjima of Lake Hamana, during the period from May to November.

Examinations of many metaphase plates of the diploid and haploid complements reveal that the diploid number of chromosomes is definitely 36 in both the male and female cells and that the haploid number is 18 in the primary and secondary spermatocytes. Figures 12 to 14 represent the diploid complexes of the male and female, and Figures 15 to 19 show the haploid groups. Thus, the number of chromosomes of this species is the same as that of the foregoing species. Further, the general configuration of chromosomes is nearly similar in the two species. As in the former species, the diploid complement of this species consists of 34 minute spheroidal elements and two prominent large-sized ones manifesting some undulations.

Now, attention should be directed again to the morphology and behaviour of the two large, elongated chromosomes just mentioned.



Figs. 12-19. Chromosomes of *Thereuopoda clunifera* (Wood). 12-13, Spermatogonial metaphases. 14, Oogonial metaphase. 15, Primary spermatocyte metaphase. 16, Primary spermatocyte metaphase, lateral view. 17, Primary spermatocyte anaphase. 18, Secondary spermatocyte metaphase. 19, Secondary spermatocyte anaphase. $\times 3800$.

They lie always in the central area of the equatorial plate surrounded by the minute ones. They show size-difference, though this is not remarkable, in the male cell (Figs. 12-13), while they are identical as to both size and shape in the female cell (Fig. 14). From this fact and in the light of the findings in the former species, it is highly probable that these two large elements are the sex chromosomes. In the male cell they are represented by the larger X and the smaller Y, although the size-difference is inconspicuous. Obviously they show an XX complex in the female cell, judging from the similarity in size and shape.

Of 18 elements observed in the first metaphase plate, 17 are ordinary bivalents of minute size, while the remaining one is very prominent because of its large size (Figs. 15-16). This is the XY complex composed of the larger X and the smaller Y in end-to-end conjugation. The XY complex manifests remarkable longitudinal splitting along its entire length. At anaphase, the XY complex segregates into two identical halves along the longitudinal splitting, thus resulting in two equal XY dyads. As the result of the first division every secondary spermatocyte produced possesses a similar complement of chromosomes, numbering 18, and made up of 17 autosomal dyads plus an XY complex (Fig. 18). The XY complex is again prominent in the second metaphase because of its very large size, being in end-to-end conjugation. At anaphase of the second division the XY complex without exception becomes separated into the X and Y elements. After segregation the X and Y migrate to the opposite poles (Fig. 19). Thus the second division results in the production of two sorts of secondary spermatocytes; the one is the X class and the other the Y class.

As mentioned above, the XY complex follows a quite similar course of division during the meiotic stages in both the former and the present species. Furthermore, the X and Y elements of the two species are nearly alike in their morphological characters. The only difference by which these two species are distinguishable lies in the point that the size-difference between the X and the Y is less in *Thereuopoda clunifera* than in *Thereuonema hilgendorfi*.

SUMMARY

The chromosomes were observed in both male and female cells of two species of chilopods, *Thereuonema hilgendorfi* and *Thereuopoda clunifera*, with special regard to the sex chromosomes. In both species the diploid number of chromosomes was observed to be 36 in cells of both sexes; the haploid number is 18 in the primary and secondary

spermatocytes. The diploid complement consists of 34 elements of spheroidal shape and two prominent very large ones showing some undulations. The fact that the two large elements manifest a size-difference in the male cell, while they are identical in size in the female cell, is satisfactorily interpreted by considering them as the sex chromosomes. They represent the X and Y chromosomes in the male cell because of the presence of a size difference, while in the female cell they show an XX complex, judging from their similarity in shape and size.

The X and Y chromosomes conjugate end-to-end in the first metaphase, forming the XY complex. The XY complex segregates equationally in the first division along its entire length through longitudinal splitting. In the second division the XY complex separates reductionally into its component elements, the X and Y. Thus the post-reductional segregation of the XY chromosomes was established in both species.

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